Methods for the Isolation and Characterization of Constituents of Natural Products. II.

Separation of Homologous Series of Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone by Thin-Layer Chromatography

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INTRODUCTION

In a previous report from this Laboratory (*1*), the preparation of homologous series of esters of primary, secondary, and tertiary aliphatic alcohols with pyruvic acid 2,6-dinitrophenylhydrazone was described. The present report concerns the separation of homologous series of these esters by thin-layer chromatography (TLC). Partition chromatography, utilizing an ultra-fine grade of Celite as a support, was selected as offering very mild conditions while still possessing high resolving power for the compounds. Both normal- and reversed-phase partition systems are described.

APPARATUS AND REAGENTS

Polyethylene glycol 400 was purchased from the J. T. Baker Company, Phillipsburg, New Jersey; Nujol (a brand of heavy mineral oil) was obtained from a local pharmacy; Micro-Cel T-38, an ultra-fine, synthetic, hydrous calcium silicate with an average particle size of 3.2 μ was obtained from the Johns-Manville Company, Baltimore, Maryland, and was dried 24 hours at 100°C before use; benzene, obtained from the Fisher Scientific Company, Silver Spring, Maryland; *n*-hexane (high purity grade) obtained from the Phillips Oil Company, Bartlesville, Oklahoma, and Acetonitrile (Baker) were redistilled. The TLC spreader was obtained from Research Specialties Company, Richmond, California. The mounting board was homemade, standard size, all aluminum; the de-

veloping tanks were standard, rectangular, high size and were purchased from the Brinkmann Company, Westbury, New York.

EXPERIMENTAL

Normal partition-chromatography. Polyethylene glycol 400 (12.5 ml) is dissolved in 60 ml of absolute alcohol in a 125-ml Erlenmeyer flask, and 15 g of Micro-Cel T-38 is added. The flask is stoppered and shaken vigorously by hand for 3-5 minutes and the slurry is spread over five 8 × 8-inch plates in the usual manner. The solvent is allowed to evaporate at room temperature until the odor of ethanol is absent and the plates are placed in a 100°C oven for an additional 5 minutes. Benzene solutions of the esters are spotted and the plate is developed with the mobile phase (hexane:benzene (85:15) saturated with the stationary phase) in an equilibrated tank lined with filter paper. Development time is approximately 45 minutes. The yellow spots may be changed to violet by placing the finished dry plate in a tank containing a wad of cotton wet with diethylamine. This procedure considerably increases the visual detection of the esters.

Reversed-phase chromatography. The reversed-phase thin-layer plates are prepared by slurrying 15 g of Micro-Cel T-38 in 90 ml of hexane and 5 ml of Nujol. The slurry is shaken for 3-5 minutes by hand and spread over four 8 × 10-inch plates. The plates are ready to use after standing approximately 15 minutes at room temperature. Benzene solutions of the esters are spotted at the origin. Approximately the top inch of the plate is scraped off and the plate is placed in the tank, scraped-side down, to equilibriate for approximately 1 hour. Care should be taken first to remove any Celite adhering to the bottom edge of the glass which might act as a wick conducting solvent from the paper lining the tank onto the main chromatographic surface. The plate is inverted after the equilibration period and developed with the mobile phase (acetonitrile:water (85:15)) for about 45 minutes. It was not found necessary to saturate the mobile phase with Nujol, highly satisfactory chromatograms being obtained without saturation.

RESULTS AND DISCUSSION

Figures 1-3 show the separations achieved for the esters of primary, secondary, and tertiary alcohols, respectively, in the normal partition system. The first 11 members of the primary alcohols are well separated. The C_{12} ester separates from the C_{11} but not from the C_{13} ester and is,

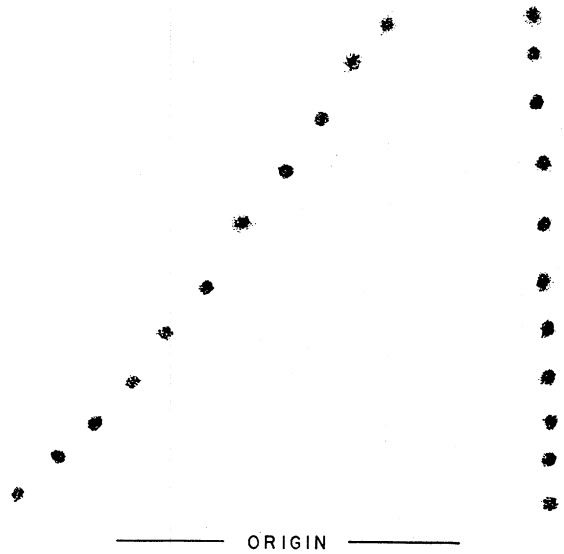

FIG. 1. Thin-layer partition chromatogram of esters of primary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_{11} through C_1 primary alcohols. Column on right represents mixture of all 11 alcohols.

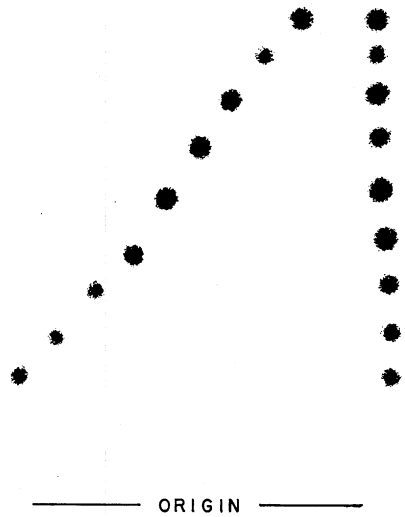

FIG. 2. Thin-layer partition chromatogram of esters of secondary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_{11} through C_3 secondary alcohols. Column on right represents mixture of all nine alcohols.

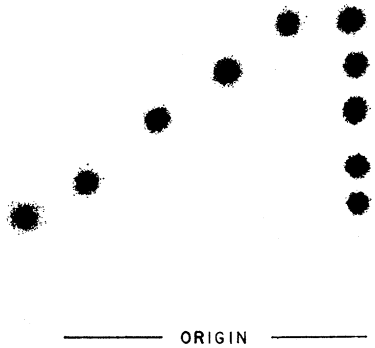

FIG. 3. Thin-layer partition chromatogram of esters of tertiary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_8 through C_4 tertiary alcohols. Column on right represents mixture of all five alcohols.

therefore, not included. The first nine members of the secondary alcohol esters (C_3 through C_{11}) separate satisfactorily as was expected from results with the primary alcohol esters. Only the C_4 through the C_8 tertiary alcohol esters were prepared and good separation of these was made.

Approximately 1.5×10^{-3} μmoles of an ester can be detected when one views the plate during chromatography. Exposure of the dry, finished plate to the vapors of diethylamine gives violet spots and increases the sensitivity about 15 times. Evaporation of the diethylamine from the plate restores the normal yellow color of the spots. This cycle can be repeated at will.

Figures 4 and 5 are reproductions of reversed-phase chromatoplates of the long-chain primary and secondary alcohol derivatives, respectively. The system separates the C_{12} through the C_{19} members and, thus, the normal partition and reversed-phase partition systems described will separate the entire homologous series of the alcohol derivatives prepared thus far in this Laboratory.

The merits of TLC have been well documented. The methods described here have been, in our hands, extremely useful. Plates are ready for use shortly after their preparation; the cost per plate is practically negligible; the layer produced is quite stable even though no binder is used; the stationary phase is uniformly distributed on the plate; the solvent front moves perfectly straight; the spots stay quite compact throughout the development.

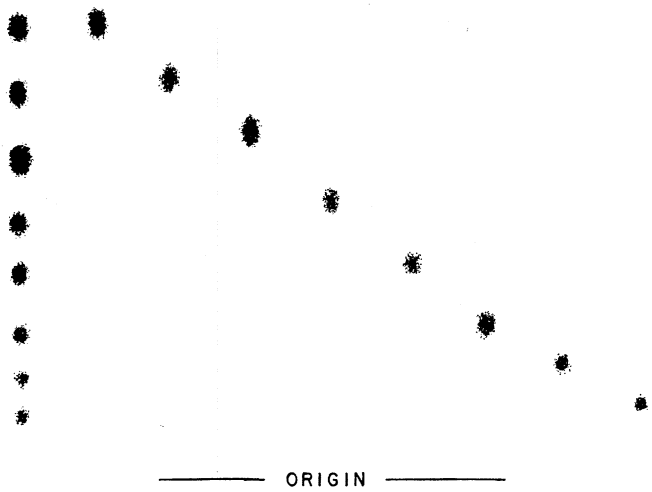

FIG. 4. Thin-layer reversed-phase partition chromatogram of esters of long-chain primary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from right to left C_{12} through C_{19} primary alcohols. Column on left represents mixture of all eight alcohols.

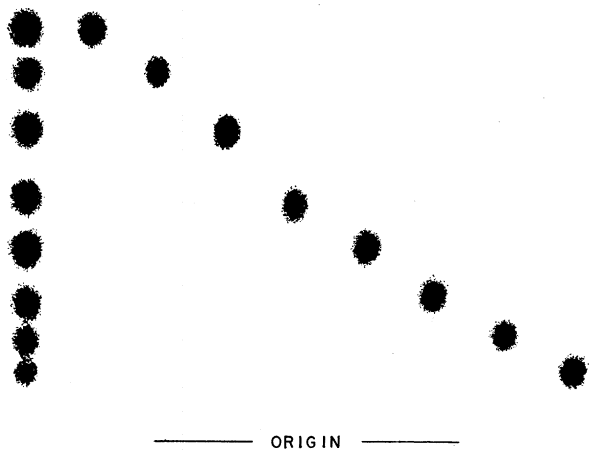

FIG. 5. Thin-layer reversed-phase partition chromatogram of esters of long-chain secondary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone. Diagonally from top to bottom C_{12} through C_{19} secondary alcohols. Column on left represents mixture of all eight alcohols.

SUMMARY

Esters of homologous series of primary, secondary and tertiary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone have been separated by thin-layer partition chromatography. The C_1 through C_{11} primary, the C_3 through C_{11} secondary and the C_4 through the C_8 tertiary alcohol derivatives are separated in the normal partition system employing polyethylene glycol as the stationary phase and hexane:benzene as the mobile phase. The C_{12} through C_{19} primary and secondary alcohol derivatives are separated by reversed-phase thin-layer chromatography using mineral oil as the stationary phase and acetonitrile-water as the mobile phase. Micro-Cel T-38 is used as the support in both systems. Approximately 1.0×10^{-4} μmoles of an ester can be detected on the plate when exposed to diethylamine vapor.

REFERENCE

1. SCHWARTZ, D. P., AND BREWINGTON, C. R., Methods for the isolation and characterization of constituents of natural products. I. Derivatives of alcohols with pyruvyl chloride 2,6-dinitrophenylhydrazone. *Microchem. J.* **11**, 430-436 (1966).